

ATTACHMENT 5**APPENDIX 4 TO SUBPART A OF PART 435 – DETERMINATION OF BIODEGRADATION OF SYNTHETIC BASE FLUIDS IN A MARINE CLOSED BOTTLE TEST SYSTEM: SUMMARY OF MODIFICATIONS TO ISO 11734:1995**

The six modifications specified in this Appendix shall apply to the determination of the biodegradability of synthetic base fluids as measured by ISO 11734:1995. These modifications make the test more applicable to a marine environment and are listed below:

1. The laboratory shall use sea water in place of freshwater media.
 - 1.1 The sea water may be either natural or synthetic. The allowable salinity range is 20-30 ppt.
 - 1.2 To reduce the shock to the microorganisms in the sediment, the salinity of the sediment's porewater shall be between 20-30 ppt.
2. The laboratory shall use natural marine or estuarine sediments in place of digested sludge as an inoculum. The VS of the sediments must be no less than 2%.
 - 2.1 Sediment should be used for testing as soon as possible after field collection. If required, the laboratory can store the sediment for a maximum period of two months prior to use. The test sediment shall be stored in the dark at 4 °C.
 - 2.2 The laboratory shall use the sediment mixing procedure specified in Appendix 3 to Subpart A of part 435 to spike the test sediment with base fluids. The final concentration will be 2000 mg carbon/Kg dry weight sediment. No less than 25 g dry weight of the spiked sediment shall be used per 125 ml serum bottle. The volume of sediment and seawater in the bottle shall be 75 ml.
3. The temperature of incubation shall be 29±1 °C.
4. The pH is maintained at the level of natural sea water, not at 7.0 as referenced in ISO 11734:1995.
5. The optional use of a trace metals solution as specified in method ISO 11734:1995 shall not be used as part of these test modifications.
6. The laboratory shall conduct the test for 275 days. The laboratory may seek approval of alternate test durations under the approval procedures specified at 40 CFR 136.4 and 136.5. Any modification of this method, beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

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